K123364

JUL 15 2013

510(k) Summary

This 510(k) summary information is being submitted in accordance with the requirement of SMDA 1990 and 21 CFR 807.92.

1) **Submitter's Name:** DIAsource ImmunoAssays
Address: Rue du Bosquet, 2 B-1348 Louvain-la Neuve, Belgium
Phone Number: +32(0)1084991
Contact Person: Napoleon Monce (Tel: 530-759-8000)
Date: May 29, 2013

2) **Name of Device:** 25OH Vitamin D Total ELISA Test

Trade Name: 25OH Vitamin D Total ELISA Test

Common Name: Vitamin D Assay

Device Classification Name: Vitamin D Test System

Product Code: MRG – Vitamin D Test System

Panel: Chemistry (75)

Regulation Number: 21 CFR 862.1825 – Vitamin D Test System – Class II

3) **Legally marketed device to which the submitter claims equivalence (Predicate Device):**
Immuno diagnostic systems 25-Hydroxy Vitamin D EIA for the quantitative determination of 25-hydroxy vitamin D and other hydroxylated metabolites in serum or plasma. K021163.

4) **Description of the device:**
The assay requires a total of 165 minutes incubation time. The DIAsource 25 OH Vitamin D Total ELISA Test is an enzyme linked immunosorbent assay to detect total 25OH Vitamin D (D2 and D3) present in human serum. During the first incubation at room temperature, 25OH Vitamin D is dissociated from binding serum proteins to fix on binding sites of a specific monoclonal antibody. After washing, a fixed amount of 25OH Vitamin D-labeled with biotin in presence of horseradish peroxidase (HRP) compete with unlabeled 25OH Vitamin D2 and 25OH vitamin D3 present on the binding sites of the specific monoclonal antibody. After another incubation at room temperature, the microtiterplate is washed to stop the competition reaction. A chromogenic solution (TMB) is added and then stopped with a Stop Solution after the last incubation period. The amount of substrate turnover is determined colorimetrically by measuring the absorbance which is inversely proportional to the total 25OH Vitamin D concentration.

5) **Intended use of the device:**
The DIAsoure 25 OH Vitamin D Total ELISA is intended for the quantitative measurement of 25-hydroxy vitamin D2 and D3 (25 OH-D2 and 25 OH-D3) in human serum. The results are to be used in conjunction with other clinical and laboratory findings to assess the Vitamin D status of a patient.

6) **Comparison with the predicate device:**
The DIAsource 25OH Vitamin D Total ELISA Test Kit was compared to a commercially available 25OH Vitamin D ELISA kit manufactured by ImmunoDiagnostic Systems (IDS) (K021163). Below is a table comparing the two kits.

Table 1: Kit Comparison

The DIAsource 25OH Vitamin D Total ELISA	IDS 25OH Vitamin D ELISA
Intended Use - For the quantitative determination of 25-hydroxyvitamin D_2 and D_3 (25-OH D_2 and 25-OH D_3) in human serum. The results are to be used in conjunction with other clinical and laboratory findings to assess the Vitamin D status of a patient.	**Intended Use** - For the quantitative determination of 25-hydroxyvitamin D (25-OH D) in human serum. The results are to be used in conjunction with other clinical and laboratory data to assist the clinician in the assessment of Vitamin D sufficiency in adult populations.
Platform - ELISA plate read on a plate reader	Same
Microtiter Plate - 96 wells coated with monoclonal anti 25OH D_2 and D_3	**Microtiter Plate** - 96 wells coated with sheep polyclonal anti 25OH D
Wash Solution Concentrate – 200x	**Wash Solution Concentrate** – 20x
Incubation Buffer – Proprietary buffer for dissociating vitamin D	Same
Conjugate Concentrate – 100x	None
HRP Concentrate – 200x	**HRP Conjugate** – Ready to Use
Conjugate Buffer – Use to dilute Conjugate and HRP Concentrate	None
Substrate – Tetramethylbenzidine (TMB)	Same
Stop Solution – Acid mixture	Same
Calibrators 0-5 - Lyopilized	**Calibrators 0-6** - Ready to Use
Controls 1-2 - Lyophilized	Same
Interpretation of Results – Standard Curve	Same
Expected Values: Deficient <10 ng/ml; Insufficient 10-29 ng/ml; Sufficient 20-100 ng/ml; Potential Toxicity >100ng/ml	Same

6(b1) Nonclinical tests:

Calibrators and Controls:
Traceability/Calibrators Value Assignment – The master calibrator stock is an ethanolic solution prepared in house by weighing 25OH Vitamin D3. The value assignment of the stock is made by UV absorbance at 254 nm using a molar extinction coefficient of 18000 L mol^{-1} cm^{-1}. The stock solution of 25OH Vitamin D is then used to make calibrators by diluting into horse serum. Calibrator values

have been determined using native serum samples that have been assayed by LC/MS-MS. The LC/MS-MS method has been validated against a reference method.

Controls Value Assignment – The mean values of 30 replicates obtained from 10 independent runs were assigned as the control target values. The controls target ranges were assigned as mean +/- 20% if the observed CV's are less than 7%; mean +/- 25% if observed CV's are 7-10%; and mean +/- 30% if the observed CV's are 10-15%.

Stability Study Protocol and Acceptance Criteria – Accelerated stability studies were performed on the kit and components. Kits and components were placed in an incubator at 37°C for 30 days and for 2 components 42 days and 56 days then tested. Following DIAsource experience that 7 days at 37°C is equivalent to six months at 4°C, 30 days ,42 days and 56 days at 37°C is equivalent to 24, 36 and 48 months, respectively. The results are accepted if they fit into 3 predefined criteria when compared to an unopened kit stored at 4°C.

These criteria are :

1. The OD of the Calibrator 0 and the Calibrator 6 have to be at +/- 50% of values obtained with the component (or kit) kept at 4°C

2. The (OD for Calibrator 1 / OD for calibrator 0) X 100 has been at +/- 15% of the value obtained with the component kept at 4°C

3. The values of the Positive and Negative controls have to be in the announced range with the component (or kit) kept at 4°C

In conclusion the stability of the kit and components was found to be 24 months with the Incubation Buffer and Conjugate Buffer at 48 months.

An expiration of 18 months at 4°C is given to the kits.

Ongoing real time stability is being conducted.

Open Vial Stability – Calibrators and Controls come in lyophilized form. Once reconstituted with DI water, they were stored at 4°C for 7 days, and frozen at -20°C for one month, two months and three months with one thawing. The results after 7 days at 4°C and after one, two, and three months at -20°C were acceptable, when compared to a new kit.

In conclusion the Calibrators and Controls can be used up to 7 days when stored at 4°C or up to three months when stored at -20°C

Precision:
Precision studies were evaluated according to the Clinical and Laboratory Standards Institute (CLSI) EP5-A Guideline.

Precision was calculated by running four serum samples at the given n value for 20 days on 3 different lots in singlicate. The results are summarized in the table below:

Intra-Assay					Inter-Assay			
Sample	n	<X> ± SD (ng/ml)	CV (%)		Sample	n	<X> ± SD (ng/ml)	CV (%)
A	24	5.6± 0.4	7.8		A	42	17.7 ± 1.3	7.4
B	35	27.4 ± 1.5	5.5		B	10	26.3 ± 1.3	4.7
C	35	43.0 ± 1.2	2.7		C	10	42.0 ± 1.9	4.5
D	24	81.2 ± 2.0	2.5		D	21	85.4 ± 7.8	9.4

The reproducibility of the assay was done by testing 60 serum samples each at 3 concentrations duplicate for five days, twice a day, at three sites with two technicians per site. The mean results are summarized in the table below:

Sample	n	ng/ml		Within-Run	Between-Run	Between-Day	Between-Tech	Between-Site	Total
1	60	25.5	SD	0.217	0.611	0.975	1.537	2.206	2.593
			CV	0.3%	0.9%	3.8%	6.0%	8.7%	10.2%
2	60	52.9	SD	0.638	1.571	1.108	2.285	4.310	5.192
			CV	0.9%	2.3%	2.1%	4.3%	8.2%	9.8%
3	60	124.8	SD	1.00	1.735	1.834	3.391	4.906	6.190
			CV	1.4%	2.5%	1.5%	2.7%	3.9%	5.0%

Limit of Detection:

The Limit of Blank (LoB), Limit of Detection (LoD), and the Limit of Quantitation (LoQ), were determined in accordance with the CLSI guideline EP17-A.

The LoB was calculated by measuring the blank several times and calculating the 95th percentile of the distribution of the test values. The LoB was calculated to be 1.69ng/ml.

The LoD was calculated by using the formula LoD = LoB + 1.65 x SDs (from the CLSI EP17-A) where SDs is the standard deviation of a low value serum. The LoD was calculated to be 2.81 ng/ml.

The LoQ was calculated by testing 5 samples of low value 10 times in different test. The mean value was put in the X axis and the CV values on the Y axis. The LoQ was calculated to be 4.32ng/ml.

Recovery:

Recovery was assessed by adding different levels of 25OH Vitamin D to samples. The results are summarized in the table below:

Recovery Test	
Added 25OH-Vit D_3 (ng/ml)	Recovery (%)

0	100
25	95
50	92
Added 25OH-Vit D_2 (ng/ml)	**Recovery (%)**
0	100
25	105
50	95

Linearity:
Two samples with concentrations known to be distributed throughout the measurable range were tested at equidistant dilutions to determine the linear range of the assay. A linear regression analysis was performed. The results are summarized in the following table:

Sample 1

Sample Dilution	Theoretical Concentration (ng/ml)	Measured Concentration (ng/ml)	Slope	Y-Intercept	R^2	Recovery (%)
1/1	96.7	96.7	1.015	-0.298	0.99	100
1/2	48.5	47.6				98.1
1/4	24.2	24.5				101.2
1/8	12.1	11.1				91.7
1/16	6.0	6.2				103

Sample 2

Sample Dilution	Theoretical Concentration (ng/ml)	Measured Concentration (ng/ml)	Slope	Y-Intercept	R^2	Recovery (%)
1/1	122.9	122.9	1.005	0.435	0.99	100
1/2	61.5	64.5				105
1/4	30.7	31.5				103
1/8	15.4	15.0				97.4
1/16	7.7	7.6				98.7

The linear range of the assay was found to be 7.7 ng/ml to122.9 ng/ml.

Time Delay:
Time delay test between the last Calibrator and sample dispensing results is shown in the following table.

	Time Delay		
	0 min (ng/ml)	10 min (ng/ml)	20 min (ng/ml)
Sample 1	27.9	30.5	30.2
Sample 2	49.5	47.5	49.0

Assay results remain accurate even when incubation buffer is dispensed 10 and 20 minutes after the Calibrator has been added in the coated wells.

Analytical Specificity:

Cross reactivity of the 25OH Vitamin D Total ELISA assay was determined by testing sera with spiked and unspiked cross reactants. The results are summarized in the following table:

Compound and Concentration	Spiked Vitamin D (ng/ml)	Unspiked Vitamin D (ng/ml)	% Cross Reaction
1,25$(OH)_2$-Vitamin D_3 at 200 ng/ml	57.3	16.7	20.3
1,25$(OH)_2$-Vitamin D_2 at 690 ng/ml	29.9	16.7	1.9
Vitamin D_3 at 200 ng/ml	22.5	16.7	2.9
Vitamin D_2 at 200 ng/ml	19.3	16.7	1.3
24,25$(OH)_2$-Vitamin D_3 at 20 ng/ml	87.9	16.7	>100
25,26$(OH)_2$-Vitamin D_3 at 4 ng/ml	31.1	16.7	>100
3-epi-25 hydroxy Vitamin D_3 at20 µg/ml	31.58	16.7	0.07
25 OH Vitamin D_3 at 10 ng/ml	26.7	16.7	100
25 OH Vitamin D_2 at 10 ng/ml	25.0	16.7	83

The effect of potential interfering substances on samples using the DIAsoure 25 OH Vitamin D Total ELISA test was evaluated. Different levels of Hemoglobin, Bilirubin, Triglyceride, Vitamin C, Bilirubin Conjugate and Unconjugated and Zemplar in serum samples were tested on samples with different 25OH Vitamin D Concentration. Our acceptance criteria was to have interference of less than 10%. The tested substances did not affect the performance of the DIAsoure 25 OH Vitamin D Total ELISA test.

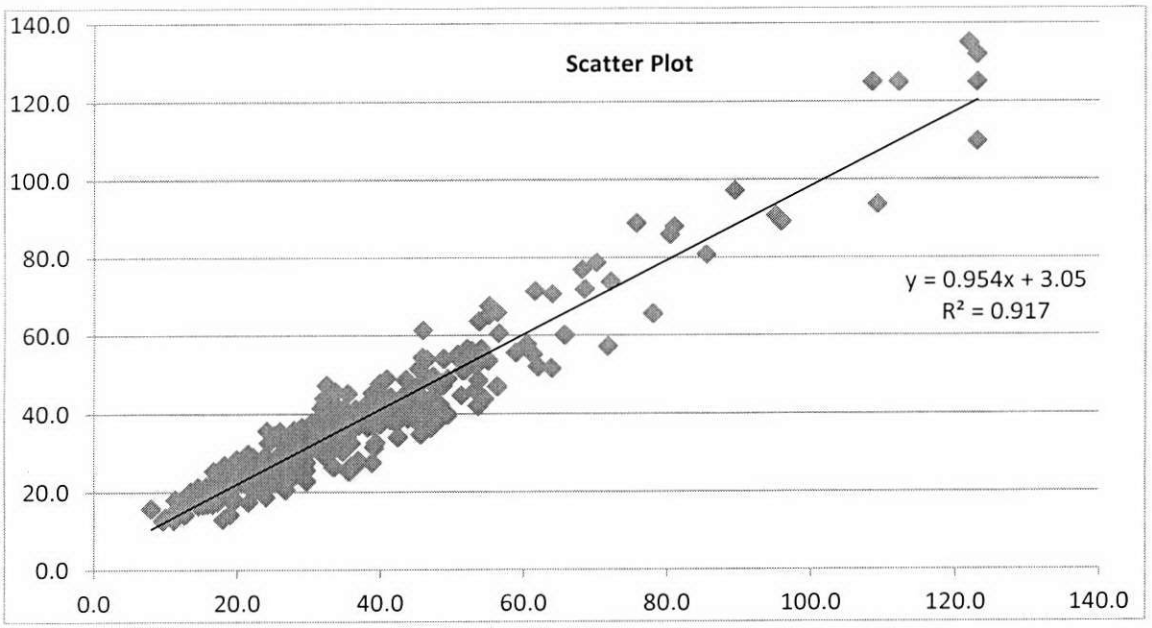
Substance	25OH Vitamin D Concentration (ng/ml)	Concentration of Interferent (mg/dL)	Mean Percent Variation
Hemoglobin	7.6	250	-0.5%
		500	
	29.3	250	
		500	
	42.5	250	
		500	
Bilirubin Conjugated	6.0	50	-3.5%
		100	
	21.5	50	
		100	
	38.6	50	
		100	

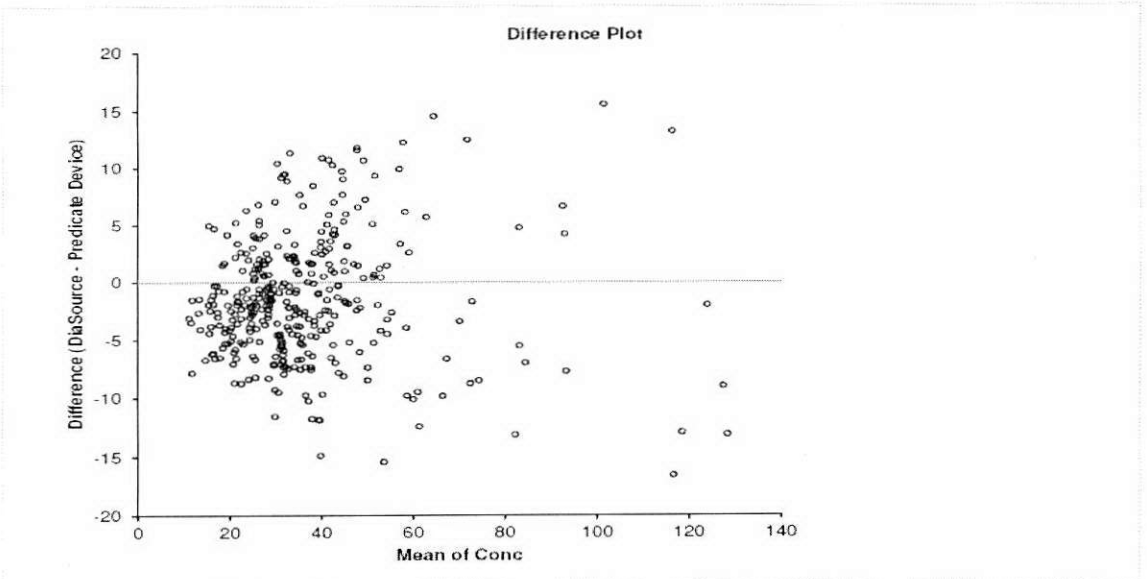
Bilirubin Unconjugated	7.6	50	2.5%
		100	
	29.3	50	
		100	
	42.5	50	
		100	
Trygliceride	7.6	7.5	-4.3%
		125	
		250	
		500	
	29.3	7.5	
		125	
		250	
		500	
	42.5	7.5	
		125	
		250	
		500	
Vitamin C	6.0	1	4.5%
		10	
		100	
	21.5	1	
		10	
		100	
	38.6	1	
		10	
		100	
Biotin	8.7	0.2	4.6%
		2	
		4	
	19.8	0.2	
		2	
		4	
	36.1	0.2	
		2	
		4	
Zemplar	17.6	$1.25x10^{-3}$	-4.3%
		$2.5x10^{-3}$	
		$5.0x10^{-3}$	
	33.5	$1.25x10^{-3}$	
		$2.5x10^{-3}$	
		$5.0x10^{-3}$	

6(b2): Clinical Comparison:

Method Comparison:

The performance of the DIAsource 25OH Vitamin D Total ELISA test was determined by conducting a correlation study tested at three different sites using a total of 356 samples. The samples were tested on both the DIAsource 25OH Vitamin D Total ELISA test and a commercially available 25OH Vitamin D ELISA test manufactured by ImmunoDiagnostics System (IDS). The results ranged from 8.0ng/ml to 123.0 ng/ml, the correlation coefficient between the two methods was 0.917, with the 95% confidence interval of 87.6% to 93.6%, a slope of 0.954 and the y-intercept of 3.05. The following plots summarizes the results:





Reference Range:

To determine the reference range, the total 25OH Vitamin D of 150 apparently healthy individuals were measured. The individual patient serum samples used were obtained from a certified commercial source (Dx Biosamples, San Diego, CA.) and were collected from an FDA Licensed Donor Center with informed consent. 50 samples were from Northern US (Pennsylvania), 50 samples were from Central US (Tennessee), and 50 samples were from Southern US (Florida).

All samples met the following criteria:

- Age between 21-90 years old.
- Subjects from three different geographical locations.
- Samples collected in the Winter season (January, February, and March)
- Subjects were not taking any vitamin D supplements
- Subjects were of different skin tones
- Subjects had no family history of parathyroid, or calcium regulatory disease.
- Subjects had no history or Kidney, Liver, Parathyroid, Calcium related disease or bariatric surgery.
- Subjects were not taking any medications known to affect absorption or catabolism of Vitamin D.

The following results were obtained:

- Ages between 20-62 years old.
- Population consisted of 75 light skin (50%) and 75 dark skin (50%).
- No subjects were taking vitamin D supplements.
- No subjects had a family history of parathyroid, or calcium regulatory disease.
- No subjects had a history of Kidney, Liver, Parathyroid, Calcium related disease or bariatric surgery.
- No subjects were taking any medications known to affect absorption or catabolism of Vitamin D.
- The following table is the summary or results:

	Florida	Tennessee	Pennsylvania	Overall
Highest Conc. (ng/mL)	88.6	71.7	54.6	88.6
Lowest Conc. (ng/ml)	6.1	4.9	5.9	4.9
Median Conc. (ng/ml)	20.8	15.9	14.3	17.2

Only Central 95% (2.5% - 97.5%) of the results observed were used.

6(b3) Conclusion:

From the data and comparison above, it is our contention that the DIAsource 25OH Vitamin D Total ELISA Test Kit is substantially equivalent to the commercially available 25OH Vitamin D ELISA kit manufactured by ImmunoDiagnostic Systems (IDS) (K021163).

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

July 15, 2013

DIAsource Immunoassays, S.A.
c/o Napoleon Monce
c/o Gold Standard Diagnostics
2851 Spafford Street
DAVIS CA 95618

Re: K123364
Trade/Device Name: 25OH Vitamin D Total ELISA Test
Regulation Number: 21 CFR 862.1825
Regulation Name: Vitamin D test system
Regulatory Class: II
Product Code: MRG
Dated: June 12, 2013
Received: June 13, 2013

Dear Mr. Monce:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801), please go to http://www.fda.gov/AboutFDA/CentersOffices/CDRH/CDRHOffices/ucm115809.htm for the Center for Devices and Radiological Health's (CDRH's) Office of Compliance. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Carol C. Benson -S for

Courtney H. Lias, Ph.D.
Director
Division of Chemistry and Toxicology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

Indications for Use

510(k) Number (if known): **K123364**

Device Name: **25OH Vitamin D Total ELISA Test**

Indications for Use:

The DIAsource 25OH Vitamin D Total ELISA Test is intended for the quantitative determination of 25-hydroxyvitamin D2 and D3 (25-OH D2 and 25-OH D3) in human serum. The results are to be used in conjunction with other clinical and laboratory findings to assess the Vitamin D status of a patient.

Prescription Use ___X___ (21 CFR Part 801 Subpart D)

And/Or

Over the Counter Use ____. (21 CFR Part 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE; CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostics and Radiological Health (OIR)

Ruth A. Chesler -S

Division Sign-Off
Office of In Vitro Diagnostics and Radiological Health

510(k) k123364